REMARKS-General

1. The amended independent claim 1 incorporates all structural limitations of the original claim 1 and includes further limitations previously brought forth in the disclosure. No new matter has been included. The amended claims 1 and 2 are submitted to be of sufficient clarity and detail to enable a person of average skill in the art to make and use the instant invention, so as to be pursuant to 35 USC 112.

2. The applicant respectfully submits that the purpose of the current amendments to claims 1 and 2 is to clearly define and directly emphasize the distinguished features of the innovative process for amplifying **sense-oriented** and **full-length version** of the mRNAs and resulting in novel composition matter of **sense-oriented full-length** RNA amplicons of the present invention.

Response to Rejection of Claims 1-3, 7-18, 20, 25, 26, and 29-35 under 35USC103

3. According to the guidance in In re Wands, 8 USPQ2d 1400 (CAFC 1988), the applicants respectfully submit that the original claims 1 and 2 are amended to fully match the claimed subject matter of the instant invention to the description of the original specifications and examples, as pursuant to 35USC112, first paragraph.

4. The amended claim 1 is narrowed to contain only the first preferred embodiment (FIG. 1) which was described in the contents of specification from page 4, last paragraph bridging to page 6, line 12; and the examples 1-5. As indicated in Example 4, its resulting evidence of amplified **sense-oriented full-length** RNAs in Figures 4a and 4b.

5. The amended claim 2 describes optional additional round(s) of amplification steps for full-length sense-oriented RNA amplicons without the need for deoxyribonucleotide tailing at the 3'-end of the first-strand cDNAs which have the homopolymeric tails incorporated at the 5'-end of the amplified sense-oriented full-length RNAs in the steps as described in the claim 1.

6. The breadth of scope of the claims is narrowed to match the first preferred embodiment (FIG. 1) based on a step-wise description as mentioned above. The narrowed Claims emphasize directly and define clearly the distinguished features of the

innovative process for amplifying **sense-oriented** and **full-length version** of the mRNAs and resulting in novel composition matter of **s nse-oriented full-length** RNA amplicons of the Present Invention. Hence, the applicants believe that the narrowed Claims would avoid the confusion of generic term of RNA amplification as often used in such other processes known to the one of ordinary skill in the art when the Present Invention was made, such as the Mallet's and Van Gelder's methods mentioned by the Examiner in the above Office Action, all of which disclose only RNA amplification for generating **PARTIAL** and **NON-full-length** RNA amplicons that are often in antisense orientation to the original mRNA sequences.

7. The applicants believe that it is necessary, in view of the amended claims 1 and 2, to outline the comparative differences among the Mallet's method, Van Gelder's method and the Present Invention as follows.

The Mallet's method teaches 1) a sequence-dependent, gene-specific amplification of specific genes or sequences, for known but **NOT** for unknown sequences or the whole RNA repertoire or transcriptome, from starting RNA material; 2) using both gene-specific 3'-primer and 5'-primer in a reverse transcription (for making first-strand cDNA)-coupled polymerase chain reaction (RT-PCR) process that applies only to specific RNA sequence amplification; 3) the amplification is done by multiple (usually 20-50) cycles of PCR with each PCR cycle provides two copies of the desired sequences; 4) obtaining the resulting amplified products in double-stranded and **NON**-full-length DNA fragment forms; and 5) the resulting products of amplified double-stranded (ds) DNA can not be used directly as template for *in vitro* protein/peptide synthesis.

The Van Gelder's Method teaches 1) a sequence-independent whole RNA transcriptome amplification, for known or unknown sequences, from starting RNA material; 2) using a 3'-Primer of an RNA promoter-linked oligodeoxythymidylate primer to perform reverse transcription for first strand cDNA and double-stranding via RNA priming or random priming, not at the far most end of the 5' region of mRNA, to build double-stranded cDNA templates with the RNA promoter driving RNA transcription in **antisense** orientation complementary to the messenger RNA (mRNA); 4) the amplification is done by limited (1-3) rounds of RNA transcription with each round provides hundreds to thousands copies of the desired sequences; 5) obtaining the

resulting amplified products in single-stranded RNA forms; 6) resulting amplified RNA amplicons being not only in orientation **antisense** (complementary) to the mRNA orientation but also of **partial length** to the original full-length mRNA sequences; and 7) the resulting amplified **partial antisense** RNA amplicons can not be used directly as template for *in vitro* protein/peptide synthesis.

The Present Invention teaches 1) a sequence-independent whole RNA transcriptome amplification, for known or unknown sequences, from starting RNA material; 2) using a 3'-Primer of an oligodeoxythymidylate primer to perform reverse transcription for first strand cDNA, building homopolymeric nucleotide tails at the VERY 3'-END of the first strand cDNA for the generation of a specific binding site at the far most 5'-end of mRNA, and then using an **external** 5'-Primer of an RNA promoter-linked complementary homopolymeric nucleotide primer for cDNA double-stranding to build double-stranded cDNA templates with the RNA promoter driving RNA transcription in **sense** orientation (the same orientation to mRNA) and **full-length** to the original mRNA sequences; 3) the amplification is done by limited (1-3) rounds of RNA transcription with each round provides hundreds to thousands copies of the desired sequences; 4) obtaining the resulting amplified products in single-stranded RNA forms; 5) resulting RNA being in orientation **sense** to the mRNA orientation and of **full length** to the original full-length mRNA sequence; and 6) the resulting amplified **sense-oriented full-length** RNA amplicons can be used directly as template for *in vitro* protein/peptide synthesis.

8. The Examiner appears to reason that since Mallet et al. teach a method whereby RNA is first subject to reverse transcription so as to generate applicants' "first-strand complementary DNAs". These cDNAs are then incubated with primers and subject to amplification so to yield applicants' "amplified RNAs", it would have been obvious to one skilled in the art to have modified the method of Mallet so to permit the transcription of DNA into RNA as Van Gelder et al. teach explicitly of conducting RNA transcription amplification of cDNA. This is, however, clearly **NOT** a proper basis for combining references in making out an obviousness rejection of the Present Invention and Claims. Rather, the invention must be considered as a whole and there must be something in the reference that suggests the combination or the modification. See Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick, 221 U.S.P.Q. 481, 488 (Fed. Cir.

1984) ("The claimed invention must be considered as a whole, and the question is whether there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination"), In re Gordon, 221 U.S.P.Q. 1125, 1127 (Fed. Cir. 1984), ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification."). In re Laskowski, 10 U.S.P.Q.2d 1397, 1398 (Fed. Cir. 1989), ("Although the Commissioner suggests that [the structure in the primary prior art reference] could readily be modified to form the [claimed] structure, "[t]he mere fact that the prior art could be modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.")

9. The applicants respectively believe that the rejection of Claims 1-3, 7-18, 20, 25, 26 and 29-35 under 35 U.S.C. 103(a) was based on unreasonable speculation of a postulated conjunction between Mallet's patent (US Patent 5,817,465) and Van Gelder's technology (PNAS, USA, March 1990, 87: 1663-1667) which speculation of conjunction is uncertain and impractical to give rise to the innovative process of generating **SENSE-ORIENTED** and **FULL-LENGTH** RNA amplicons as clearly defined and directly emphasized in the amended and narrowed Claim 1 and Claim 2 of the Present Invention. In any case, even combining Mallet et al. and Van Gelder et al. would not provide the Present Invention as claimed – a clear indicia of nonobviousness. Ex parte Schwartz, slip op. p.5 (BPA&I Appeal No. 92-2629 October 28, 1992), ("Even if we were to agree with the examiner that it would have been obvious to combine the reference teachings in the manner proposed, the resulting package still would not comprise zipper closure material that terminates short of the end of the one edge of the product containing area, as now claimed.") That is modifying or conjuncting Mallet et al. with Van Gelder et al., as proposed by the Examiner, would not generate a homologous oligonucleotide sequence tail at the 3'-end of the first-strand cDNA useful for binding by an external homologous complementary oligonucleotide sequence of the 5'-primer containing the RNA promoter region for making sense-oriented full-length double-stranded cDNAs as templates for RNA transcription amplification creating **SENSE-ORIENTED** and **FULL-LENGTH** RNA amplicons of the original mRNAs. Such externally added 5'-promoter is ABSOLUTELY required and capable of not only generating sense RNA amplicons as in same orientation as the original mRNA but also assuring the full-length integrity of the original mRNA. The translation-start codon for

protein synthesis is usually located in the 5'-end region of the mRNA. The amplified RNA amplicons produced by the instant invention preserves the intact 5'-end of the mRNA for protein synthesis.

Even in the view of combination of the Mallet's method with the Van Gelder's method, as reasoned by the Examiner in the above Office Action, the combined Mallet's and Van Gelder's methods would not give rise to the innovative process of the Present Invention of generating SENSE-ORIENTED and FULL-LENGTH RNA amplicons: 1) by using a homopolymeric tailing step of the 3'-end of the first-strand cDNA, **which step is taught or disclosed NEITHER by Mallet's method NOR by Van Gelder's method**, 2) for binding by an **external** 5'-primer comprising a specific polynucleotide complementary to the homopolymeric tails and an RNA promoter region, **which external 5'-primer is taught or disclosed NEITHER in Mallet's method NOR in Van Gelder's method**, and 3) resulting in double-stranded cDNA templates comprising RNA promoter region in the sense-orientation at location corresponding to the **5'-far most end** of the mRNA, **which double-stranded cDNA template for full 5' to 3'-end RNA transcription is taught or disclosed NEITHER by the Mallet's method NOR the Van Gelder's method**. In another words, neither Mallet's method nor the Van Gelder's method teaches the homopolymeric tailing of the 3'-end of the first cDNA, thus, the combination or conjunction of Mallet's and Van Gelder's methods would **NOT** produce the innovative process and resulting **SENSE-ORIENTED FULL-LENGTH** RNA amplicons as explicitly taught, disclosed and claimed by the Present Invention.

10. In response to Point 4 of the Examiner's rejection, Mallet et al. disclose an RT-PCR amplification process for amplifying gene-specific RNA sequence into amplified gene-specific double-stranded DNA sequence. As discussed in column 5 and recognized by the Examiner, the Mallet's RT-PCR method teaches using a **3'-primer** for reverse transcription and then using both 3'- and 5'-primers for PCR that can contain a non-specific oligonucleotide tail which may contain a promoter region. However, the 5'-primer can be designed to locate at any where in between the 5' and 3' regions, BUT NOT at the **FAR MOST 5'-END** of mRNA, because there is no way to warrant a non-specific (random) primer to specifically match the various kinds of 5'-ends of a mRNA repertoire. Unlike 3'-poly(A)+ tail of mRNA, there is no homopolymeric region at the far most 5'-end of mRNA. The 3'-primer of the Mallet's method cannot be designed to

situated at the **FAR MOST END** of the 5' region of mRNA as well, otherwise, there would be no reverse transcription for first-strand cDNA synthesis. On the other hand, the 3'-primer in the Van Gelder's method uses the oligodeoxythymidylate linked with an RNA promoter for making the first-strand cDNA starting **ONLY** from the 3'-poly (A)+ tails of mRNA, but **ABSOLUTELY NOT** from any region at the 5'-end of mRNA. Additionally, both Van Gelder's and Mallet's methods do not teach using a specific external homopolymeric oligonucleotide tailed 5'-primer for cDNA double-stranding that may contain a promoter region. In column 6, bridging to column 7, Mallet et al does disclose making first strand cDNA using gene-specific 3'-primer and performing gene-specific amplification using a second gene-specific 5'-primer, however, using PCR amplification which makes two copies per each PCR cycles. The Mallet's method in view of Van Gelder's method does not teach using *in vitro* RNA amplification for amplifying sense-oriented full-length mRNA sequences, because there is no homopolymeric region at the 5'-end of mRNA for the binding of a specific homopolymeric oligonucleotide tailed 5'-primer for cDNA double-stranding. The Present Invention provides such a uniform homopolymeric binding region at the far most 5'-end of mRNA by terminally tailing the 3'-end of the first strand cDNA. Without this artificial homopolymeric tail at the 5'-end of mRNA, no specific amplification of mRNA can start from the far most 5'-end of the mRNA and thus, no sense-oriented full-length RNA amplicons can be made without the teaching of present invention.

11. In response to Point 5 of Examiner's rejection, the Mallet's method does teaches one or more rounds of amplification in column 7, last paragraph, alas, by the PCR amplification process which can only make two-fold doubling per each PCR round, instead of hundreds to thousands folds of amplification by *in vitro* transcription (IVT) by RNA polymerase as used in both Van Gelder's method and our Present Invention. For making millions-fold amplification, the PCR amplification process requires typically 20 to 50 rounds of amplification, while the IVT RNA amplification requires only two to three rounds of IVT RNA transcription. It is well known to the one of ordinary skill in the art that the PCR amplification is fundamentally different process from the IVT RNA transcription. The PCR cycle requires temperature cycling from high denaturing temperature (typically at 94°C) to low annealing temperature (typically at 37°C to 72°C) and primer extension (typically at 72°C) of the 5'- and 3'-primers, the IVT RNA transcription is an isothermal process at typically 37°C and requires an RNA promoter

rather than the 5'- and 3'-primers. The 5'-and 3'-primers in Van Gelder's method and our Present Invention are designed for reverse transcription and cDNA double-stranding, but not for PCR amplification. However, the differences between Van Gelder's method and our Present Invention is that Van Gelder's method teaches the use of 3'-primer for RNA promoter incorporation, while the instant invention teach the use of an external 5'-primer for the same purpose. It is easy to use 3'-primer for RNA promoter incorporation since most of mRNAs carry 3'-end poly(A)+ tail. In order to use an external 5'-primer for RNA promoter incorporation, a uniform specific oligonucleotide tail must be artificially created to the 3'-end of the first strand cDNA (equal to the 5'-end of mRNA). Both Mallet's and Van Gelder's methods do not teach this tailing reaction which is necessary for generating a specific binding site at the 5'-end of mRNA.

12. In response to Point 6 of Examiner's rejection, the applicants agree with the Examiner that all of the methods mentioned can start with the same starting material of mRNA, or total RNA.

13. In response to Point 7 of Examiner's rejection, the Examiner argued favorably for the Present Invention that Mallet's method does not teach using a specific primer that contains an oligodeoxythymidylate region due to the fundamental limitation of sequence-dependent amplification of the Mallet's method and nor it discloses synthesizing RNA from the cDNA amplicons.

14. In response to Point 8 of Examiner's rejection, the applicants respectfully agree in part with the Examiner that Van Gelder's method teaches using a first 3'-primer containing oligodeoxythymidylate linked with an RNA promoter and teaches using *in vitro* RNA transcription for amplification. However, the Van Gelder's method specifically requires the use of oligodeoxythymidylate primer containing the RNA promoter region for priming from the Poly (A)+ tail of mRNA for making the first strand cDNA, thus, the RNA promoter is situated in **antisense** orientation to the mRNA. The amplified RNA amplicons from the cDNA so obtained are in **antisense** orientation and can NOT be in sense orientation as the Present Invention does. It must be noted that Van Gelder et al. had defined his invention product as amplified **antisense RNA (aRNA)** as shown by the Abstract, Materials & Methods, Figure 1 and Results of his PNAS publication (March 1990). Therefore, the Van Gelder's method discloses a method that places orientation restriction upon which strand of the cDNA is being amplified.

15. In response to Point 9 of Examiner's rejection, the applicants respectfully disagree with the Examiner that the first primer or 3'-primer of Van Gelder's method of oligodeoxythymidylate linked with a promoter region could replace the primer of the Mallet's RT-PCR method for gene-specific amplification, wherein the Mallet's primer has to contain a gene-specific sequence region at the 3'-distal end and the gene-specific primer sequence cannot be substituted by the oligodeoxythymidylate sequence which is naturally non-gene-specific. Because a non-specific primer binds randomly to both strands of cDNA, none of ordinary skill in the art at the time of the Present Invention was made can predict the transcription of RNA polymerases from either sense or antisense orientation or both. Transcription reaction by RNA polymerases from both sense and antisense directions will cause the stall of whole transcription machinery in the middle of cDNA template and therefore fail the possibility for any kinds of RNA amplification. Thus, the Mallet's RT-PCR does not teach or disclose sequence-independent RNA amplification needed for whole mRNA transcriptome amplification. Also, the full-length RNA synthesis is impractical to Mallet's method as well because there is no homopolymeric region in the far most 5'-end of mRNA, whereas the homopolymeric poly(A)+ tail of mRNA is at its 3'-end. Although the Van Gelder et al. overcome Mallet's problem by means of reverse transcription from 3'-poly(A)+ tail of mRNA using a promoter-linked oligodeoxythymidylate primer, both methods still only teach the modification of the first 3'-primer but not the 5'-primer as the Present Invention does. Even in view of Van Gelder's method using random non-specific primers that contain an RNA promoter region, it is impossible for anyone of ordinary skill in the art to warrant the binding of non-specific random primers to the various 5'-ends of a mRNA repertoire. In conclusion, it is impractical and uncertain to make a conjunction of Mallet's and Van Gelder's methods for sense-oriented full-length RNA amplification

16. In further response to Point 9 of Examiner's rejection, the examiner's postulation of a possible conjunction between Mallet's and Van Gelder's methods has the following uncertainty issues to deal with. Firstly, both Mallet's and Van Gelder's methods do not teach the generation of a specific annealing site at the VERY 3'-end of the first strand cDNA for the binding of a promoter-linked oligopolymeric primer for generating FULL-LENGTH double-stranded cDNA templates with the RNA promoter sequence located at the 5'-end of the sense-oriented second cDNA strand of the double-strand cDNA for making sense-oriented FULL-LENGTH mRNA amplicons. Secondly, both Mallet's and

Van Gelder's methods do not teach an approach for synthesis of a sense-oriented and full-length RNA library or RNA transcriptome which is the DESIRED END PRODUCTS of the Present Invention. Simple conjunction of Mallet's and Van Gelder's methods with RNA promoter internally situated in an antisense orientation, is simply uncertain and impractical for one of ordinary skill in the art to produce the desired end products of sense-oriented full-length RNA amplicons without the teaching of the Present Invention.

17. In further response to Examiner's rejections, Van Gelder et al. do not teach the use of promoter-linked homopolymeric tail including oligodeoxythymidylate region for cDNA double-stranding to form the second-strand sense-oriented cDNA. It is widely known that the first strand of cDNA product which is generated by reverse transcription from mRNA is an antisense strand, while the second strand cDNA which is formed by double-stranding of the first cDNA is a sense strand. The instant invention incorporates a "specific" external promoter-linked homopolymeric primer during cDNA double-stranding for making the second strand cDNA in the sense orientation, which is completed under a totally different enzymatic condition and mechanism in comparison to Van Gelder's method. Although Mallet et al. teach the use of a non-specific oligonucleotide tailed primer that can contain a promoter region, however, Mallet's non-specific oligonucleotide tail is at the 5'-distal of the first 3'-primer while the 3'-distal of the first 3'-primer is gene-specific, the conjunction of both Mallet's and Van Gelder's methods still does not provide a specific homopolymeric tail in the sense orientation needed for mRNA amplification. There is no evidence in either Mallet's or Van Gelder's method that provides a mean to generate a specific binding region in the 5'-end of mRNA for promoter incorporation and full-length mRNA synthesis. Lack of a specific homopolymeric tail for the promoter incorporation into the sense orientation of double-stranded cDNA template is one of common problems to both Van Gelder's and Mallet's methods. Such specific homopolymeric tail for the promoter incorporation into the 5'-end sense orientation is important not only for the maintenance of full-length mRNA conformation but also the amplificational fidelity of whole RNA library integrity.

18. In further response to Examiner's rejection, Mallet's method is to incorporate promoter during polymerase chain reaction (PCR) doubling process rather than one-cycle reaction of cDNA double-stranding. PCR is a multiple-cycle (or round) (typically in 20 to 50 cycles) reaction of DNA polymerization while cDNA double-stranding can be a single-cycle DNA polymerization. Because Mallet et al. propose their design of various

non-specific promoter-linked primers binding to an internal sequence of RNA or relative cDNA, the completion of cDNA double-stranding in the promoter region will require at least two cycle of PCR reaction, of which the first cycle fill in 3'-end of its complementary strand cDNA from 5'-matched primer region and then the second cycle fill in the 5'-non-matching promoter region from the reverse site of the complementary strand cDNA in the 5' to 3' direction. Since there is neither a template for the double-stranding of 5'-mismatched promoter region in the first cycle DNA polymerization reaction nor an enzymatic reaction to fill in non-matching promoter region from 3' to 5' direction, Mallet's method do not teach the means for promoter incorporation using one-cycle cDNA double-stranding DNA polymerization reaction except PCR (multiple cycles of DNA polymerization reaction). On the other hand, the Present Invention provides a specific external 5'-end tail for the binding of promoter-linked primer through its specific homopolymeric region and the double-stranding of 5'-non-matching promoter region can be completed within one cycle of DNA polymerization reaction by simply primer and strand extension elongation of the specific binding site of the external 5'-end tail. Therefore, it would have been obvious to one of ordinary skill in the art to understand that the Present Invention is a **non-PCR-based** RNA amplification method, whereas Mallet's method is a **PCR-based** method as described in their patent. Based on the above differences in reaction procedures and mechanisms, Mallet's method in conjunction with Van Gelder's method can not teach the same protocol of the instant invention.

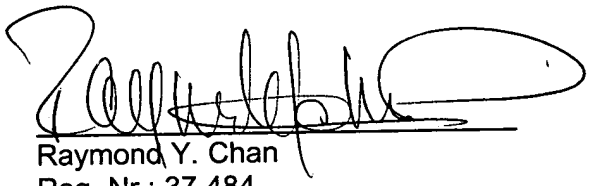
19. Taken together, Mallet et al. may teach the use of antisense first 3'-primer containing a non-specific 5'-distal oligonucleotide tail that can contain a promoter region for RT-PCR, but not the use of a specific homopolymeric oligonucleotide tail, while the Van Gelder et al. teach the use of antisense first 3'-primer of a promoter-linked oligodeoxythymidylate primer during reverse transcription for antisense RNA synthesis, but not during cDNA double-stranding procedure which is essential for sense mRNA amplification. The conjunction of these two methods do not lead to the Present Invention, because both methods do not solve the problem of lack of a specific binding region in the 3'-end of the first-strand cDNA for RNA promoter incorporation at the FAR MOST 5'-end of the second-strand cDNA for mRNA synthesis, indicating that an additional terminal tailing reaction is needed. Furthermore and most importantly, any conjunction of Mallet's and Van Gelder's methods would not produce the DESIRED

END PRODUCTS of **sense-oriented** and **full-length** RNA amplicons, in particular an entire sense-oriented and full-length mRNA transcriptome, of the instant invention.

20. In summary, conjunction of Mallet's and Van Gelder's methods, as speculated by the Examiner, is uncertain and impractical to the one of ordinary skill in the art at the time of the Present Invention was made to give rise to a prior art of the Present Invention. Therefore, the basis for decision of claim rejection to Claims 1-3, 7-18, 20, 25, 26 and 29-35 under 35 U.S.C. 103(a) in view of the conjunction of Mallet's and Van Gelder's methods was unfounded.

21. In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the rejection are requested. Allowance of claims 1 and 2 at an early date is solicited.

Respectfully submitted,



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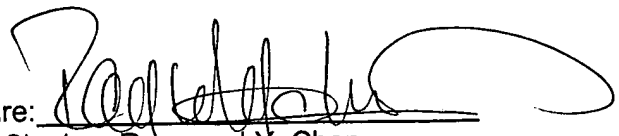
Signature: 
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